COMPOSITIONS AND METHODS OF USING CAPSID PROTEIN FROM FLAVIVIRUSES AND PESTIVIRUSES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application Serial Number 60/237,885, filed October 4, 2000, incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to the use of the capsid protein from West Nile virus, and capsid and other proteins from other viruses including viruses of the *Flavivirus* and *Pestivirus* genuses, to induce the death of cells by apoptosis, and to vaccines and diagnostics for West Nile virus and other viruses including *Flavivirus* and *Pestivirus* infections. The invention also relates to methods of screening for antiviral compounds by identifying compounds that selectively inhibit the ability of capsid protein to induce apoptosis.

BACKGROUND OF THE INVENTION

West Nile virus (WNV) infection has recently emerged in temperate regions of Europe and North America, presenting a threat to humans, horses, and birds. The most serious manifestations of WNV infection is fatal encephalitis. WNV, originally isolated in the West Nile District of Uganda in 1937, is a *Flavivirus* of the *Flaviviridae* family, having a size of 40 - 60 nm, an enveloped, icosahedral nucleocapsid, and a positive-sense, single-stranded RNA genome of 10,000 - 11,000 bases. For a recent review of WNV, *see* Holloway, 2000, Outbreak not contained. West Nile virus triggers a reevaluation of public health surveillance, Sci. Am., 282:20, 22, which is incorporated herein by reference. Reviews of the viruses in the *Flaviviridae*

family are provided in the following references: Neyts *et al.*, 1999, Infections with *Flaviviridae*, Verh. K. Acad. Geneeskd. Belg., 61:661-697, discussion 697-699; Leyssen, *et al.*, 2000, Perspectives for the treatment of infections with *Flaviviridae*, Clin. Microbiol. Rev., 13:67-82; Sherlock, 1999, The hepatic *Flaviviridae*: summary, J. Viral. Hepat.,6 Suppl.1:1-5; and Fields, Knipe, & Howley, eds., Fields Virology (3rd ed.) Vols. I & II, Lippincott Williams & Wilkins Pubs. (1996), each of which is incorporated herein, in its entirety, by reference.

There is a need for improved methods of prophylactic and therapeutic treatment of *Flavivirus* and *Pestivirus* infection. There is a need for improved methods of inducing cell death and of treating diseases characterized by hyperproliferating cells.

SUMMARY OF THE INVENTION

The present invention provides methods of inducing the death of cells. The methods of the invention comprise the step of contacting cells with an amount of a *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, effective to induce cell death. According to some embodiments of the invention, the *Flavivirus* capsid protein, or functional fragment thereof, is the capsid protein, or functional fragment thereof, of West Nile virus (WNV). According to some embodiments of the present invention, cells are contacted with *Flavivirus* or *Pestivirus* capsid protein, or a functional fragment thereof, in order to induce the cells to die. According to some embodiments of the present invention, a nucleic acid molecule that comprises a sequence which encodes a *Flavivirus* or *Pestivirus* capsid protein, or a functional fragment thereof, is introduced into the cells. Expression of the sequence that encodes the *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, results in the production of the *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, within the cell, causing the cell to die. According to some embodiments of the present invention, the sequence which encodes the *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, is operably linked to regulatory elements which are necessary for expression of the sequence in the cell. According to some embodiments of the present invention, the nucleic acid molecule is DNA. According to some embodiments of the invention, the cells are tumor cells.

The present invention provides methods of identifying compounds that inhibit the ability of *Flavivirus* or *Pestivirus* capsid protein, or functional fragments thereof, to induce apoptosis. Methods of the invention comprise the steps of (a) contacting cells, in the presence of a test compound, with an amount of *Flavivirus* or *Pestivirus* capsid protein, or a functional fragment

thereof, sufficient to induce a measurable amount of apoptosis in the cells, and (b) comparing the amount of apoptosis that occurs when the test compound is present with the amount of apoptosis that occurs when the test compound is absent. The present invention relates to a method of identifying compounds that inhibit WNV capsid protein, or functional fragments thereof, from inducing apoptosis in cells that comprises the steps of (a) contacting cells, in the presence of a test compound, with an amount of WNV capsid protein, or a functional fragment thereof, sufficient to induce a measurable amount of apoptosis in the cells, and (b) comparing the amount of apoptosis that occurs when the test compound is present with the amount of apoptosis that occurs when the test compound is absent. According to some embodiments, the measuring step of the method is accomplished by detecting the presence of apoptosis-related markers, including phosphatidylserine (PS) of the cellular membrane, and free 3'-hydroxy termini in DNA.

The present invention relates to pharmaceutical compositions that comprise a *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, and a pharmaceutically acceptable carrier. According to some embodiments of the present invention, the pharmaceutical composition comprises WNV capsid protein, or a functional fragment thereof, and a pharmaceutically acceptable carrier.

The present invention relates to pharmaceutical compositions that comprise a nucleic acid molecule that comprises a sequence which encodes a *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, and a pharmaceutically acceptable carrier. According to some embodiments of the present invention, the pharmaceutical composition comprises a nucleic acid molecule that comprises a sequence which encodes a *Flavivirus* or *Pestivirus* capsid protein, or a functional fragment thereof, that is operably linked to regulatory elements which are necessary for expression of the sequence in the cell. The present invention relates to pharmaceutical compositions that comprise a nucleic acid molecule that comprises a sequence which encodes WNV capsid protein, or a functional fragment thereof, and a pharmaceutically acceptable carrier. According to some embodiments of the present invention, the pharmaceutical composition comprises a nucleic acid molecule that comprises a sequence which encodes WNV capsid protein, or a functional fragment thereof, that is operably linked to regulatory elements which are necessary for expression of the sequence in the cell. According to some embodiments of the present invention, a pharmaceutical composition comprises a nucleic acid molecule that is DNA.